

HYBRIDIZATION ASSAY USING
SELF-QUENCHING FLUORESCENCE PROBE

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ABSTRACT

10 A hybridization assay is provided which uses an oligonucleotide probe
which includes a fluorescent reporter molecule and a quencher molecule
capable of quenching the fluorescence of the reporter molecule. The
oligonucleotide probe is constructed such that the probe exists in at least one
single-stranded conformation when unhybridized where the quencher molecule
is near enough to the reporter molecule to quench the fluorescence of the
reporter molecule. The oligonucleotide probe also exists in at least one
conformation when hybridized to a target polynucleotide where the quencher
molecule is not positioned close enough to the reporter molecule to quench the
fluorescence of the reporter molecule. By adopting these hybridized and
20 unhybridized conformations, the reporter molecule and quencher molecule on
the probe exhibits different fluorescence signal intensities when the probe is
hybridized and unhybridized. As a result, it is possible to determine whether the
probe is hybridized or unhybridized based on a change in the fluorescence
intensity of the reporter molecule, the quencher molecule, or a combination
25 thereof. In addition, because the probe can be designed such that the quencher
molecule quenches the reporter molecule when the probe is not hybridized, the
probe can be designed such that the reporter molecule exhibits limited
fluorescence until the probe is either hybridized or digested.